

# Temperature- and Force-Induced $\beta$ -Sheet Unfolding in an Exactly Solvable Model

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## Abstract

The stability of a  $\beta$ -sheeted conformation and its transition into a random coil are studied with a 2D lattice biopolymer model. At low temperature and low external force, the polymer folds back and forth on itself and forms a  $\beta$ -sheet. Our analytical calculation and Monte Carlo simulation reveal that a co-operative  $\beta$ -sheet-random coil transition takes places when the temperature or force is increased, with a dramatic decrease in the contact number. These predictions are in good agreement with experiments on titin protein. This transition is not a real phase-transition, indicating that backbone hydrogen-bonding alone is unable to stabilize a distinct  $\beta$ -sheet phase.

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Single-molecule manipulation techniques were used by Gaub and coworkers and several other groups [1, 2] to study the unfolding of proteins in real time. A co-operative “all-or-nothing” transition between globular and randomly coiled states was observed at a pulling force of  $\sim 10^2$  piconewtons. A stretched coil segment can be modelled as a wormlike chain of persistence length  $\sim 10^{-1}$  nanometers [1, 2], and several (atomic-level) molecular dynamics (MD) simulations have studied the initial stage of unfolding and the final stage of refolding (see, e.g., [3, 4]). Monte Carlo (MC) simulations [5] of lattice models also have been used to study the unfolding kinetics. The protein used in the experiments, namely titin immunoglobulin domains, is rich in  $\beta$ -sheets [1, 2], and numerical work demonstrated that the transition to a random coil was initiated by the rupture of only a few hydrogen bonds between adjacent  $\beta$ -strands [3]. Theoretically, however, it is still unclear why the globule-coil transition is a highly co-operative process, and why there are dramatic differences between the unfolding behavior of helix-dominated and  $\beta$ -sheet-dominated proteins [6].

An issue of current interest is to study the generic statistical mechanics of biopolymer  $\beta$ -sheet states. In this paper, the deformation and unfolding of  $\beta$ -sheets are addressed within an exactly-solvable 2D model. Because of the attractive interaction between monomers along the chain, the polymer could fold back and forth on itself, thereby maximizing its contact energy. However, a  $\beta$ -sheet, which is a relatively ordered structure and is favorable in energy, is deficit in configurational entropy compared with an irregular coil configuration. Because of this competition between energy and entropy, it is demonstrated by our analytical and simulational calculations that, when the temperature or the external force approaches some critical value, a small variation in temperature or force could lead to a dramatic change in the polymer’s microscopic configuration. This behavior corresponds well both with experimental observations and MD simulations on titin. However, we suggest that this highly co-operative transition is not a phase-transition in the strict sense; the nonspecific hydrogen-bonding interactions between the backbone molecules of a protein alone are hence unlikely to be able to stabilize a distinct  $\beta$ -sheet phase.

The mathematical model is constructed as follows: Consider a biopolymer of  $N$  identical units (monomers) located on a square lattice (Fig. 1). The distance between two consecutive monomers is  $a_0$ , and to facilitate the calculation the chain is assumed to be directed, namely the bond between monomers  $i$  and  $i + 1$  can not point to the  $-\mathbf{z}_0$  direction (Fig. 1). If two monomers with indices  $i$  and  $i + m$  ( $m \geq 3$ ) occupy nearest neighbor lattice sites, an

attractive energy of strength  $\epsilon$  is gained and these two monomers are then “in contact”. Each monomer can be in one of three states, with respectively zero, one, or two contacts. By folding back and forth on itself, the polymer can gain contact energy, and a segment of  $m$  columns in which there is at least one pair of monomers in contact between any two adjacent columns is referred to as a “ $\beta$ -sheet”. Two  $\beta$ -sheet segments are separated by a “coil segment” of  $m$  columns ( $m \geq 0$ ), in which all the monomers are free of contacts (Fig. 1).

We first discuss the free energy of the  $\beta$ -sheet state. Under the action of a force  $f$  along direction  $\mathbf{z}_0$ , the energy of a  $\beta$ -sheet of  $m$  columns is  $-\epsilon \sum_{i=1}^{m-1} v(l_i, l_{i+1}) - mfa_0$ , where  $v(l_i, l_{i+1}) = l_i - 1$  (if  $l_i \leq l_{i+1}$ ) and  $l_{i+1} - 1$  otherwise;  $l_i$  is the number of monomers in the  $i$ -th column. The partition function (PF)  $Z_\beta(n)$  of a  $\beta$ -sheet of length  $n$  is

$$Z_\beta(n) = \sum_{m=2}^{[n/2]} \sum_{l_1=2} \cdots \sum_{l_m=2} \delta_{l_1+\dots+l_m}^n b^m \prod_{i=1}^{m-1} a^{v(l_i, l_{i+1})}, \quad (1)$$

where  $\delta_i^j = 1$  ( $i = j$ ) and 0 (otherwise);  $a = \exp(\beta\epsilon)$ ,  $b = \exp(\beta fa_0)$ , and  $\beta = 1/T$ ,  $T$  being the temperature (the Boltzmann constant is set to 1).

There can be many-body interactions among the monomers in a  $\beta$ -sheet and a contact pair can be formed between two remote monomers along the chain, consequently, direct evaluation of  $Z_\beta(n)$  for a large system is difficult. To circumvent this, we define an auxiliary function  $T_m(\zeta, k) = \sum_{l_1=2} \cdots \sum_{l_m=2} \zeta^{kl_1} a^{v(l_1, l_2)-l_1} \prod_{i=2}^{m-1} (\zeta/a)^{l_i} a^{v(l_i, l_{i+1})} (\zeta/a)^{l_m}$  for  $m \geq 2$  and  $T_1(\zeta, k) = \zeta^{2k}/a(a - \zeta^k)$ . One finds

$$T_m(\zeta, k) = \frac{\zeta^{2k} T_{m-1}(\zeta, 1)}{a(1 - \zeta^k)} + \frac{\zeta^k (1 - a) T_{m-1}(\zeta, k+1)}{a(a - \zeta^k)(1 - \zeta^k)}. \quad (2)$$

Define  $L_\beta(\zeta, k) = \sum_{m=1}^{\infty} b^m T_m(\zeta, k)$ , and from the recursive relation Eq. (2) one obtains the following two equations: (1)  $L_\beta(\zeta, 2) = A(\zeta) + B(\zeta)L_\beta(\zeta, 1)$  and (2)  $L_\beta(\zeta, 2) = H(\zeta) + J(\zeta)L_\beta(\zeta, 1)$ . After eliminating  $L_\beta(\zeta, 2)$  from these two equations, one finds that

$$L_\beta(\zeta, 1) = \frac{A(\zeta) - H(\zeta)}{J(\zeta) - B(\zeta)}. \quad (3)$$

In Eq. (3),  $A(\zeta) = \zeta(1 - \zeta)/(a - 1)$ ,  $B(\zeta) = (a - \zeta)(b\zeta^2 + a\zeta - a)/b(a - 1)\zeta$ ,  $H = (b/a)[\zeta^4/(a - \zeta^2) + \sum_{i=3}^{\infty} \prod_{j=2}^{i-1} \phi(\zeta, j)\zeta^{2i}/(a - \zeta^i)]$ , and  $J = (b/a)[\zeta^4/(1 - \zeta^2) + \sum_{i=3}^{\infty} \prod_{j=2}^{i-1} \phi(\zeta, j)\zeta^{2i}/(1 - \zeta^i)]$ , where  $\phi(\zeta, j) = b(1 - a)\zeta^k/a(a - \zeta^k)(1 - \zeta^k)$ .

Both  $H(\zeta)$  and  $J(\zeta)$  are convergent when  $\zeta \in [0, 1]$ . When  $\Delta = 1 - \zeta \rightarrow 0^+$ , our analytical and numerical calculations suggest that  $A(\zeta) - H(\zeta) \rightarrow \exp(-b/a\Delta)f_1(\Delta)$  and

$J(\zeta) - B(\zeta) \rightarrow \exp(-b/a\Delta)f_2(\Delta)$ , with  $f_1(\Delta)/f_2(\Delta) \rightarrow b/a(a-1)$ . Here  $f_1(\Delta)$  and  $f_2(\Delta)$  are two polynomials of  $\Delta$  which contain terms with negative powers of  $\Delta$ .

The grand partition function (GPF) of the  $\beta$ -sheet,  $G_\beta(\zeta) = \sum_{n=4} (\zeta/a)^n Z_\beta(n)$ , is nothing but  $L_\beta(\zeta, 1) - b^2\zeta^2/a(a-\zeta)$ .  $G_\beta(\zeta)$  could be calculated based on Eq. (3) at given  $\zeta$ . It keeps increasing as  $\zeta$  approaches  $\zeta_\beta (< 1)$  from 0 and is divergent at  $\zeta_\beta$ , where  $\zeta_\beta$  is the smallest positive root of the equation  $J(\zeta) - B(\zeta) = 0$  (see Fig. 2). The free energy density of a  $\beta$ -sheet segment equals to  $\rho_\beta = k_B T \ln(\zeta_\beta/a)$ . The divergence of  $G_\beta$  at  $\zeta_\beta$  indicates the absence of a distinct  $\beta$ -sheet phase.

Armed with the partition function of a  $\beta$ -sheet, we proceed to investigate its stability and the configurational transition of the whole polymer system. To this end, one needs to know the free energy of a coil segment [7]. The total PF of a coil segment of length  $n$  under the action of an external force ( $f$ , incorporated in the parameter  $b$ ) is expressed as

$$Z_{\text{coil}}(n) = 2 \sum_{m=1}^n Z_{\text{coil}}^s(n, m) b^m + \sum_{m=1}^n Z_{\text{coil}}^m(n, m) b^m \quad (4)$$

and  $Z_{\text{coil}}(0) = 1$ . In this expression,  $Z_{\text{coil}}^s(n, m)$  is by definition the total number of allowed configurations for a coil of length  $n$  whose two ends are separated by  $m$  lattice steps along  $\mathbf{z}_0$  and whose last column has only a single monomer;  $Z_{\text{coil}}^m(n, m)$  is defined similarly, but with the last column containing at least two monomers. The coefficient 2 in Eq. (4) appears because the first column of a  $\beta$ -sheet following a coil segment that ends with a column containing a single monomer can have *two* possible orientations (Fig. 1).

One finds  $Z_{\text{coil}}^s(n, m) = Z_{\text{coil}}^s(n-1, m-1) + Z_{\text{coil}}^m(n-1, m-1)$ , and  $Z_{\text{coil}}^m(n, m) = Z_{\text{coil}}^s(n, m+1) + Z_{\text{coil}}^s(n-2, m-1)$ , with  $Z_{\text{coil}}^s(0, m) = \delta_m^0$ ,  $Z_{\text{coil}}^m(0, m) = Z_{\text{coil}}^m(1, m) = 0$ ,  $Z_{\text{coil}}^m(2, m) = \delta_m^1$ , and  $Z_{\text{coil}}^m(n, 1) = 1 - \delta_n^1$ . With these preparations the GPF of the coiled state is calculated to be

$$G_{\text{coil}}(\zeta) = \sum_{n=0}^{\infty} \zeta^n a^{-n} Z_{\text{coil}}(n) = \frac{a(a-\zeta)(a+b\zeta)}{a^3 - a^2(b+1)\zeta - b^2\zeta^3}, \quad (5)$$

It follows that the free energy density of a coil segment equals  $\rho_{\text{coil}} = k_B T \ln(\zeta_{\text{coil}}/a)$ .  $\zeta_{\text{coil}}$  is the point where  $G_{\text{coil}}(\zeta) = +\infty$ :  $\zeta_{\text{coil}} = \ln[(a/b)((\sqrt{\lambda} + b/2)^{1/3} - (\sqrt{\lambda} - b/2)^{1/3})]$  with  $\lambda = (b+1)^3/27 + b^2/4$ .

In the lattice model, any configuration of the polymer is a chain of  $\beta$ -sheets and random coils, with the two conformations occurring alternately (Fig. 1). The total PF is then

$$Z(N) = \sum_{s=0}^{[N/4]} \sum_{i_0=0} \sum_{j_1=4} \sum_{i_1=0} \cdots \sum_{j_s=4} \sum_{i_s=0} \sum_{j_0=0}$$

$$\delta_{i_0+j_0+\sum_{k=1}^s(i_k+j_k)}^N \prod_{k=0}^s [Z_\beta(j_k)Z_{\text{coil}}(i_k)], \quad (6)$$

Finally the GPF of the whole system may be calculated:

$$G_{\text{total}}(\zeta) = \sum_{N=0}^{\infty} \zeta^N a^{-N} Z_{\text{total}}(N) = \frac{[1 + G_\beta(\zeta)]G_{\text{coil}}(\zeta)}{1 - G_\beta(\zeta)G_{\text{coil}}(\zeta)}. \quad (7)$$

Because of the facts that  $G_\beta(\zeta_\beta) = +\infty$  and  $G_{\text{coil}}(\zeta_{\text{coil}}) = +\infty$ , it is always possible to find a root ( $\zeta_{\text{total}}$ ) of the equation

$$G_{\text{coil}}(\zeta_{\text{total}})G_\beta(\zeta_{\text{total}}) = 1 \quad (8)$$

in the range of  $0 < \zeta_{\text{total}} < \min(\zeta_\beta, \zeta_{\text{coil}})$ . Consequently, the free energy density of the whole system is neither that of the  $\beta$ -sheet state nor that of the coil state:  $\rho_{\text{total}} = k_B T \ln(\zeta_{\text{total}}/a)$ . There is no singularity in the total free energy expression, and hence no real phase-transition process. Almost forty years ago, it was revealed by intensified theoretical investigations that the protein  $\alpha$ -helix-coil transition is not a phase-transition, provided that only the main-chain hydrogen-bonding interactions are considered (see, e.g., [8, 9]); we are surprised to find that the same conclusion holds for the  $\beta$ -sheet transition. Although there is no strict phase-transition between  $\beta$ -sheet and random coil in the model, our analytical and simulational results presented below indicate that, when temperature and the external force are low enough the polymer is essentially in the form of a  $\beta$ -sheet, and there is an rapid change in the polymer's total extension and its total number of contacted pairs when the temperature or the force is increased.

The total contact number  $\sigma$  (scaled by  $N$ ) could be calculated by  $\sigma = 1 - \partial \ln(\zeta_{\text{total}})/\partial \ln a$  and the total extension  $\ell$  (scaled by  $Na_0$ ) could be calculated by  $\ell = -b \partial \ln(\zeta_{\text{total}})/\partial b$ . When there is no external force ( $f = 0$ ), the temperature-contact number profile and the temperature-extension profile are shown in Fig. 3. And in Fig. 4 the relationship between force and extension and between force and contact number are shown at a fixed temperature of  $T = 0.591\epsilon$ . When the temperature and the force is small,  $\zeta_{\text{total}}$  approaches  $\zeta_\beta$  (the divergent radius of  $G_\beta$ ) and its value is difficult to be numerically determined in much precision through Eq. (8). Therefore, the analytical calculation in Fig. 3 is shown only for  $T \geq 0.8\epsilon$  and that in Fig. 4 for  $f \geq 0.6\epsilon/a_0$ . In Figs. 3 and 4 the data obtained by a MC simulation is also shown. The Monte Carlo simulation is performed with a polymer size of  $N = 1024$  (the results for  $N = 5000$  are essentially the same as the data shown here).

There are three kinds of elementary updates of configurations: (i) one monomer is moved from one column to another column or stacked on the right-hand side of the last column; (ii) the positions of two columns are exchanged; (iii) the direction of one column is changed by  $180^\circ$ . Special attention is paid to make sure that ergodicity and detailed balance are obeyed. Each simulational data is obtained through  $10^9$  MC steps (it takes about 35 hours in a Compaq station). The energetically most favorable state is a  $\beta$ -sheet whose width and height both equal to  $\sqrt{N}$ . Therefore, in the numerical simulation data shown in Figs. 3 and 4 for a chain of  $N = 1024$ , one has  $\sigma \leq 0.935$  and  $\ell \geq 0.0313$ . For an infinite system,  $\sigma \rightarrow 1$  and  $\ell \rightarrow 0$  as  $T \rightarrow 0$ .

Figure 3 indicates that when  $f = 0$  and  $T < 0.75\epsilon$  almost all the monomers of the polymer are in the  $\beta$ -sheet state. At  $T \simeq 0.75\epsilon$  there is a plateau in the  $T$ - $\sigma$  and  $T$ - $\ell$  profiles, suggesting that a co-operative transition into random coiled structure takes place. During this process, the monomers lose contacts and the polymer is extended. As temperature is further increased, the configuration of the polymer becomes essentially random and there are only small changes of the total extension and the total contact number. Similar co-operative  $\beta$ -sheet-coil transition is observed when the temperature is fixed but the external force keeps increasing. At  $T = 0.591\epsilon$  Fig. 4 indicates the transition takes place at  $f \simeq 0.5\epsilon/a_0$ . In the experimental investigations of Gaub and coworkers [1] a co-operative globule-extended coil transition is observed for the  $\beta$ -sheet dominated protein titin; and in a later experiment of Fernandez and coworkers [2] it was suggested that the force-induced denaturation of protein titin should follow the same paths as the temperature-induced denaturation of this protein. The present theoretical work seems to confirm both of these experimental observations: a co-operative transition from  $\beta$ -sheet to extended coil will occur either by increase the environmental temperature or by increase the mechanical force acting on the two ends of the polymer.

Conformational transitions in single biopolymers have attracted the interest of theorists for quite a long time, and the introduction of simple mathematical models have brought a deep understanding of the issues of the protein  $\alpha$ -helix-to-coil transition[9], double-stranded DNA melting [10],  $\beta$ -hairpin formation in oligopeptides[11], and RNA secondary structure denaturation by heat [12] or by force [13], etc.. The current work extends these efforts to a new model system, namely,  $\beta$ -sheet formation in biopolymers. It suggests that a co-operative  $\beta$ -sheet-coil transition will happen at certain temperature or mechanical stress. To make it

more realistic, other effects such as the sequence heterogeneity and the interactions between adjacent layers of  $\beta$ -sheets [3] could be considered. On the other hand, the results presented here show that even without these complications the structural transition of a  $\beta$ -sheet is “two-state-like”.

Recently, the MC work in [14] suggested that pulling a collapsed polymer in 2D leads to a co-operative transition into an extended random coil, and the MC work in [15] suggested this process actually may be a continuous transition. The present effort is also complementary to these MC simulations.

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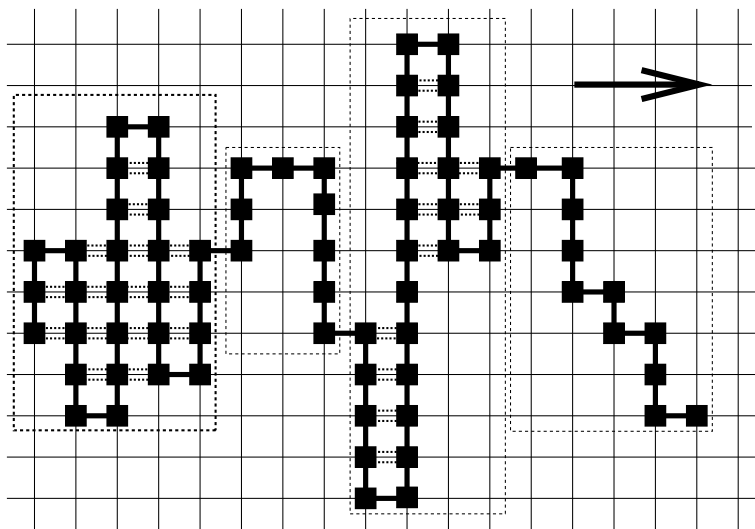


FIG. 1: A model directed biopolymer. Black squares are monomers and the arrow denotes the  $\mathbf{z}_0$  direction. The configuration shown here has two  $\beta$ -sheets and two coils.

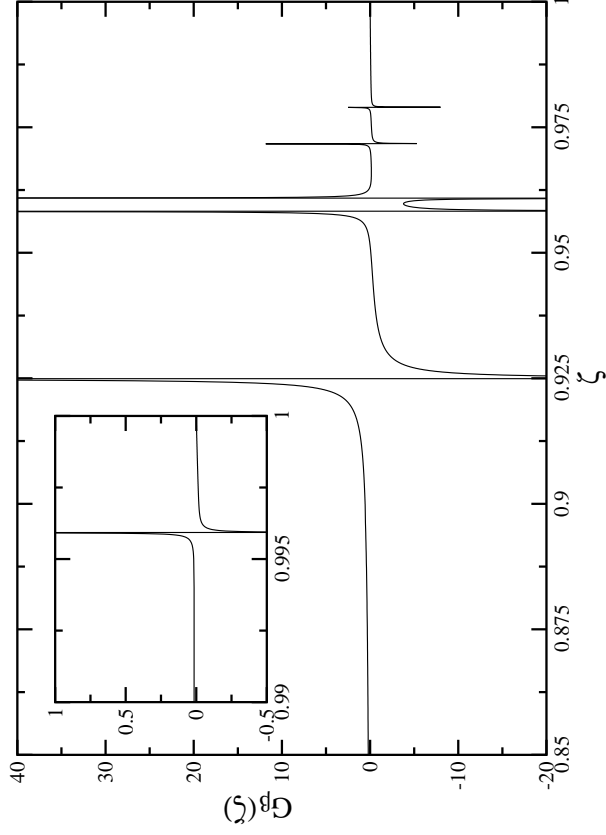


FIG. 2: The value of the partition function  $G_\beta(\zeta)$  as its divergent radius  $\zeta_\beta$  is approached. The temperature is set to  $T = 0.590928\epsilon$  and the force is  $f = 0.73866\epsilon/a_0$  or  $f = 0$  (the inset).

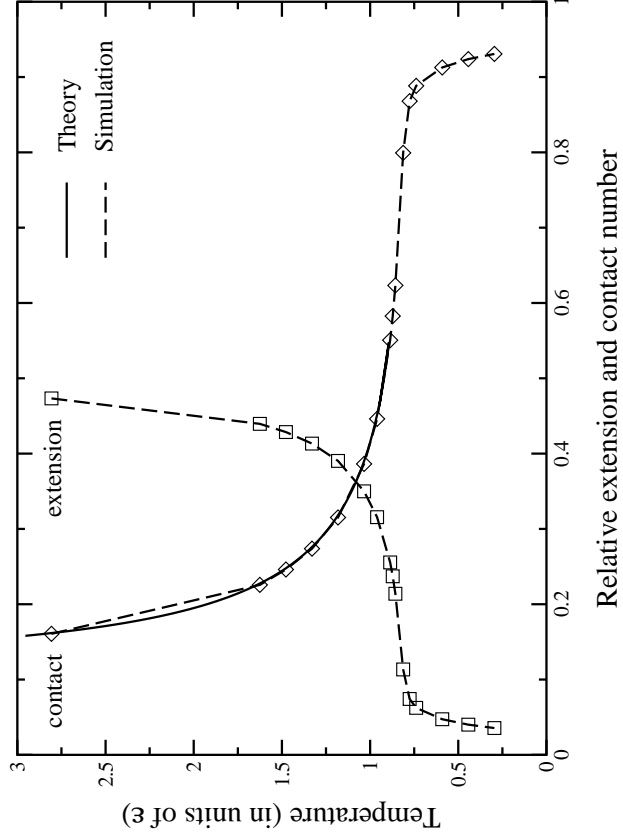


FIG. 3: Dependence of the contact number and the extension on the temperature at zero force. Symbols are MC simulation data performed for  $N = 1024$ .

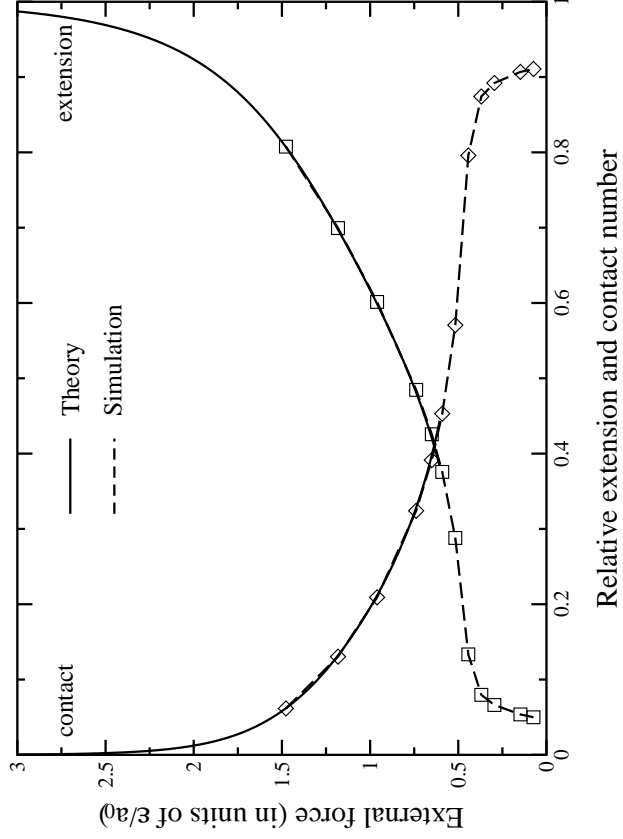


FIG. 4: Dependence of the contact number and the extension on the external force at fixed temperature  $T = 0.590928\epsilon$ . Symbols are MC simulation data performed for  $N = 1024$ .